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Low dose gamma irradiation effects on *Clostridium botulinum* inoculated turkey frankfurters containing various sodium chloride levels

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Low dose gamma irradiation (0, 0·5 and 1·0 Mrad) at two irradiation temperatures (+ 1°C and −30°C) of turkey frankfurters containing various levels of sodium chloride (NaCl; 3·25%, 2·5% or 1·5%) or 1·5% NaCl plus 0·4% sodium tripolyphosphate (TPP) was evaluated for its effect on C. botulinum *toxin production in frankfurters held at 27°C. A radiation dose of 0·5 Mrad or greater was sufficient to inhibit botulinal toxin production for 40 days in inoculated frankfurters containing 2·5% or greater concentrations of NaCl, regardless of the irradiation temperature. Neither 0·5 Mrad nor 1·0 Mrad inhibited toxin production in products containing 1·5% NaCl with or without TPP. NaCl should be reduced in processed meats with care; gamma radiation treatments cannot totally compensate for NaCl reduction.*

Introduction

Sodium reduction has been recommended as one of the steps to reduce hypertension and symptoms associated with coronary heart disease and renal failure (Pearson and Wolzak 1982). Daily dietary sodium intake varies from 3900 to 4700 mg per person, which is 10 to 15 times greater than the minimum adult requirement (IFT 1980).

Even though processed meat products contribute only about 15% of the total sodium to our daily intake (IFT 1980), some products have high sodium concentrations per serving which is a consumer concern. Consequently, the meat industry is looking for ways to reduce the sodium content of some processed meat products.

Sodium chloride (salt) has three major functions in meat products: solubilize proteins to provide desired texture, provide and enhance flavor, and inhibit pathogens (Ingram and Kitchell 1967, Kastner and Kropf 1986). Any reduction in salt may have adverse effects on those three functions. The use of ingredients that could enhance the stability and flavor of reduced salt products has been under investigation and was reviewed by Maurer (1983) and Terrell (1983) in poultry and red meat products, respectively.

Sensory evaluation studies have shown that a slight decrease in NaCl concentration can still provide accept-

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able product texture and flavor. Sofos (1983) reported that a NaCl reduction of 20% (from 2·5% to 2·0% NaCl) in frankfurters was not judged inferior by a taste panel.

Phosphates can be used to compensate for loss of texture, meat bind, water holding capacity, and flavor caused by salt reduction (Sofos 1986). According to Shults et al. (1972), phosphates act synergistically with NaCl in processed meats. Seman et al. (1980) reported that bologna made with 1·25% NaCl and 0·4% phosphate was equivalent to the control with 2·5% NaCl. The same trend was shown by Matlock et al. (1984); phosphate addition enhanced sensory and physical evaluation scores of reduced salt pork sausage.

Sodium chloride also has an important role in inhibiting microbial pathogens in processed meat products by reducing water activity (IFT, 1980). Therefore, NaCl reduction requires special attention as was stressed by Sofos (1984) in his review of the antimicrobial effects of sodium and other ions in foods. Several investigators demonstrated the importance of salt on product safety. Roberts (1973) found that 250 ppm sodium nitrite were needed to control *C. botulinum* growth in the presence of 2% NaCl, pH 6; when NaCl concentration was doubled to 4% NaCl, only 150 ppm sodium nitrite were needed for *C. botulinum* control. Pivnick and Barnett (1965) reported that increasing the NaCl level from 2·2% to 3·0% resulted in inhibition of *C. botulinum* toxin production. No toxin was produced in bologna with 3·0% NaCl stored for a month at 30°C, whereas with 2·2% NaCl toxin was produced in 1 week. Tanaka (1982) indicated that a reduction of salt in pork wieners from 3·5% to 2·0% reduced the amount of time needed for botulinal toxin to develop from 2·5 weeks to 5 days. Both the bologna and wieners were nitrite-containing products. It is likely that a significantly higher NaCl level would be needed to produce an equivalent preservative effect in a non-nitrite product.

Irradiation is one of the most promising ways to help preserve heat sensitive foods. The efforts made in this area during the last three decades have been reviewed by the Institute of Food Technologists (IFT 1983). Irradiation has been successfully used in meats and meat products to partially replace various food preservatives. For example, irradiated ham, bacon, and corned beef have been produced with much less nitrite than is commonly used and were indistinguishable in flavor from commercially produced products containing high levels of nitrite (Wierbicki and Brynjolfsson 1979).

A beneficial aspect of using sub-freezing temperatures during gamma radiation of foods is the decrease in off-flavor formation that might be initiated by the irradiation process (Josephson 1983). However, there is a trade off between protection of sensory properties at low temperatures and bacterial inactivation which is lessened at a given radiation dose when the temperature is reduced (Grecz et al. 1971). They evaluated temperatures between −196°C to 95°C and showed that *C. botulinum* spores became increasingly resistant to ionizing radiation as the temperature was decreased. The same also held true for a number of other bacteria. The range of *D*-values obtained in various irradiation experiments was summarized by Kreiger et al. (1983).

Exposure to gamma radiation at various doses will destroy or injure living bacteria cells and spores. Spore injury can result in a change to either the germination or outgrowth system. Sublethal damage to *C. botulinum* as a result of irradiation, heat, or chemical treatments results in spores which are more

sensitive than uninjured spores to agents such as NaCl (Chowdhury et al. 1976), nitrites (Roberts and Ingram 1966), and antibiotics (Labbe and Duncan 1970, Flowers and Adams 1976, Rowley et al. 1983). Mechanisms of spore injury have been proposed by Barach et al. (1974), Flowers and Adams (1976), and Rowley et al. (1983).

The objectives of this study were to determine the relationships between salt concentration and irradiation dose and temperature needed to inhibit botulinal toxin production in turkey frankfurters.

Material and Methods

Treatments

Sixteen frankfurter treatments (Table 1) were prepared in the University of Wisconsin Food Research Institute biohazard area. Three sodium chloride (NaCl) levels (3·25, 2·5 and 1·5%) and one level of 1·5% NaCl plus 0·4% tripolyphosphate (TPP) were compared with each other at two gamma radiation dose levels (0, 0·5 and 1·0 Mrad) and two irradiation temperatures (+1°C and −30°C). Only the 2·5% NaCl treatment, representing the most commonly used NaCl level in this type of product (Maurer 1983, Sofos 1983), was tested at all irradiation dose and temperature combinations. There were two trials (A and B), but not all treatments were duplicated in both trials. Comparisons between trials were made based on treatments appearing in both trials.

Ingredients

Frankfurters were made with mechanically deboned turkey meat (MDTM) which contained 14·0% protein, 13·5% fat, 70·5% moisture and 1·2% ash (AOAC 1975). The common ingredients in the frankfurters were 2% corn syrup solids, 1% dextrose, 0·25% white pepper, 0·07% nutmeg, 0·05% sodium erythorbate, 0·015% sodium nitrite and 0·5% liquid smoke (Milwaukee Seasoning Laboratories, Germantown, Wisconsin). NaCl (Columbus Chemical, Inc., Columbus, Wisconsin) levels and TPP (Stauffer Chemical, Inc., Washington, Pennsylvania) varied as specified earlier. TPP was chosen because it is one of the most popular phosphates used in the meat industry (Everson 1985).

Product manufacture

A nonvacuum bowl cutter (Hobart, model 84142, Troy, Ohio) was used to mix the ingredients and to emulsify the batter. The MDTM was chopped first, followed by spore inoculum addition and mixing for 1 min; then salt, spices, and liquid smoke were added. Salt was added last in order to delay salt-soluble protein extraction and to insure good distribution of the spores before the batter became too sticky. Final chopping temperature did not exceed 6°C.

Emulsions were stuffed into cellulose casings (26 mm diam; Union Carbide, Chicago, Illinois) using a hand-cranked stuffer, and linked every 11 cm. The frankfurters were first rinsed with tap water (10°C), then heat processed in a forced air oven (Blue M, model POM 133B-1, Blue Island, Illinois). The oven temperature was gradually increased from 50°C to 79°C and the products were heat processed for 1·5 h to a final internal temperature of 69°C. After cooking, the frankfurters were cooled by immersing in cold tap water. Following overnight storage in a 2°C cooler, the frankfurters were peeled and vacuum packaged. The packaged frankfurters were dipped into 400 ppm chlorine and ethanol solutions, and then frozen at −30°C.

A pH measurement, in duplicate, was made on the cooked frankfurters using a Beckman pH meter (Model 3500) with a Futura comb electrode.

Spore inoculum

The *C. botulinum* spore inoculum used was a composite of equal numbers of five strains each of type A and B (56A, 62A, 69A, 77A, 90A, 53B, 113B, 213B, 13983B and Lamanna-akra B). The target inoculum was 5×10^3 spores g^{-1} of frankfurter emulsion. All raw batters were inoculated with the same volume of spore suspension.

Irradiation

On the day following packaging, the frankfurters were transported frozen in dry ice to the irradiation facility at the USDA, ARS, Eastern Regional Research Center, Philadelphia, Pennsylvania. The packaged frankfurters received radiation doses of either 0·5 or 1·0 Mrad at temperatures of +1°C or −30°C in a self-contained, cesium-137 irradiator with a strength of 132,000 Ci and a dose rate of 10 krad min^{-1}. The two temperatures represent two practices by the food industry.

Table 1. The effect of NaCl reduction, radiation dose and temperature, and tripolyphosphate (TPP) addition on *C. botulinum* toxin production in turkey frankfurters

Trt. No.	Trial	NaCl %	TPP 0·4%	Radiation Mrad	Radiation °C	pH	Days at 27°C: 1	2	3	4	5	6	7	8	9	10	15	20	30	40	50	60
							(No. of toxic samples out of 3)															
1	A	3·25		0	—	6·52	—[a]		—		—		—	—	2	3						
2	A			0·5	1	6·50	—		—		—		—		—		—	—	—	—	—	—
3	A			1·0	1	6·51	—		—		—		—		—		—	—	—	—	—	—
4	A	2·5		0	—	6·54	—	—	—	—	3											
	B					6·53	—	—	—	3												
5	A			0·5	1	6·55	—			—		—		—		—				—	—	—
	B					6·50	—		—	—		—		—		—		—	—	2		
6	A			1·0	1	6·56	—		—	—		—		—		—				—	—	—
	B					6·52	—		—	—		—		—		—		—	—	—		
7	B			0·5	−30	6·53	—		—	—		—		—		—		—	—	—		
8	B			1·0	−30	6·55												—	—	—		
9	A	1·5		0	—	6·56	—	—	1	3												
10	A			0·5	1	6·58	—	—	—	3												
11	A			1·0	1	6·58	—	—	—	3												
12	B	1·5	0·4	0	—	6·66			3													
13	B		0·4	0·5	1	6·65			1	2	3											
14	B		0·4	0·5	1	6·60	—	—	—	2	3											
15	B		0·4	1·0	−30	6·61	—	—	—	3												
16	B		0·4	1·0	−30	6·64	—	—	—	—	3											

[a] No sample out of three tested was found toxic.

One is irradiation above freezing applied to products susceptible to detrimental textural changes if frozen, and the other is irradiation while frozen to inhibit microbial activity in foods which are not as sensitive to freezing. Ferrous sulfate/cupric sulfate ($FeSO_4/CuSO_4$) dosimeters were used to measure the absorbed doses.

Toxicity testing

Spore enumeration was by a 5-tube most probable number (MPN) method, botulinal toxin presence was detected by a mouse bioassay test, and confirmatory testing was as described earlier (Barbut et al. 1986).

Spore survival

Bacterial spore numbers were estimated on two samples per treatment with the non-irradiated samples serving as the controls. The MPN method described previously (Barbut et al. 1986) and the egg yolk medium of Hauschild and Hilscheimer (1977) were used for spore enumeration.

Results and Discussion

The average spore count per gram of *C. botulinum* in the cooked emulsions before irradiation was $4{\cdot}6 \times 10^2$ and $5{\cdot}4 \times 10^2$ for trials A and B, respectively.

NaCl reduction

Sodium chloride reduction in non-irradiated poultry frankfurters decreased the time for *C. botulinum* toxin production to occur. A NaCl reduction from 3·25% to 2·5% and to 1·5% resulted in detection of the first toxic samples after the 9th, 4th and 3rd day, respectively (Table 1; treatments 1, 4, and 9). Those data are in general agreement with results by Pivnick and Barnett (1965), Tanaka (1982) and Barbut et al. (1986).

Irradiation dose and temperature

At the 3·25% NaCl level a radiation dose of 0·5 Mrad inhibited botulinal toxin production up to 2 months (treatment 2). This represented an improvement of at least six-fold over the corresponding non-irradiated samples (treatment 1).

At the 2·5% NaCl level, 0·5 Mrad at 1°C delayed toxin production up to 40 days in trial B and inhibited toxin production up to 2 months in trial A (treatments 5A and B). Exposure to gamma radiation increased the time before toxic samples were detected by a factor of 10 (4 days vs 40 days; treatments 4B vs 5B). Applying radiation doses of 0·5 Mrad or 1·0 Mrad at −30°C also prevented toxin production up to 40 days when the experiment was terminated (treatments 7B and 8B). No protective effect on *C. botulinum* toxin production was apparent due to the lower irradiation temperature.

At the lower NaCl level of 1·5%, representing a 40% NaCl reduction from the commonly used level, radiation doses of 0·5 or 1·0 Mrad did not inhibit botulinal toxin production. A 1-day difference in the time of first toxin detection was noted when comparing the non-irradiated to irradiated samples (treatments 9 vs 10 and 11). However, this 1-day difference is not nearly as significant as the inhibition due to the same radiation dose in the 2·5% NaCl frankfurters.

Phosphate

The addition of 0·4% TPP had no effect on toxin production. All 1·5% NaCl plus TPP treatments became toxic by the 5th day. Irradiation delayed toxin production by about 1 day over that observed in the non-irradiated frankfurters (treatments 9–16). The pH of TPP treatments was slightly higher compared to NaCl only treatments. This was expected and has been previously reported by other investigators (Shults et al. 1972, Seman et al. 1980, Maurer 1983). However, that slight pH difference did not affect toxin production. These results are in agreement with the findings of Barbut et al. (1986) where only sodium acid pyrophosphate possessed some antibotulinal toxin properties when compared to TPP

and hexametaphosphate addition in non-irradiated turkey frankfurters (Barbut et al. 1986).

Spore survival

The number of spores surviving after irradiation at 1°C (2·5% NaCl treatment) was estimated at 140 and 50 g^{-1} of frankfurter for the 0·5 and 1·0 Mrad doses, respectively. At a −30°C irradiation temperature, slightly more spores survived. The non-irradiated 2·5% NaCl frankfurters contained 460 spores g^{-1}.

No viable spores were found in one of the frankfurter treatments which allowed no toxin production within 1 month (treatment 3A, 3·25% NaCl, 1·0 Mrad). Two samples out of three tested in the 2·5% NaCl treatment were found toxic on the 40th day indicating that at least one spore in each of those treatments was viable but delayed in growing for an extended period of time, most probably due to irradiation injury and the presence of NaCl. The spore(s) finally repaired, developed, reached maturity, and produced toxin. Based on these observations, it is postulated that irradiation damaged the spores making it more difficult for them to germinate in the presence of higher levels of NaCl.

It was shown that a low radiation dose of 0·5 Mrad killed or injured *C. botulinum* spores so they could no longer tolerate the stress of NaCl; the recovery of injured spores and their ability to produce toxin decreased with the higher concentrations of NaCl in turkey frankfurters. A 0·5 Mrad radiation dose to turkey frankfurters containing 2·5% NaCl inhibited toxin production for at least 40 days under optimal conditions for toxin production. A radiation dose of 1·0 Mrad was not sufficient to delay toxin production in products containing 1·5% NaCl. Therefore, changes in NaCl formulation should be done with care; the levels of gamma radiation studied here cannot totally compensate for NaCl reduction.

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